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     (FILE 'HOME' ENTERED AT 14:03:19 ON 14 JAN 2003)

     FILE 'MEDLINE, CANCERLIT, EMBASE, BIOSIS, BIOTECHDS' ENTERED AT 14:03:35
     ON 14 JAN 2003
L25          123 S STROMAL AND GENETICALLY MODIFIED
L26           41 S L25 AND GENE THERAPY
L27           20 DUP REM L26 (21 DUPLICATES REMOVED)
L28        74718 S OBESE
L29        25713 S OB
L30        95474 S L29 OR L28
L31        63756 S GENETICALLY MODIFIED OR EX VIVO
L32          181 S L31 AND L30
L33           13 S L32 AND GENE THERAPY
L34        74718 S OBESE
L35            0 S OBESE FACTOR
L36          774 S STROMAL AND GENE THERAPY
L37       444693 S EX VIVO OR IMPLAN?
L38          209 S L37 AND L36
L39           97 DUP REM L38 (112 DUPLICATES REMOVED)

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L27 ANSWER 20 OF 20 MEDLINE DUPLICATE 11
AN 93362170 MEDLINE
DN 93362170 PubMed ID: 8356600
TI Cell transplantation of genetically altered cells on biodegradable polymer scaffolds in syngeneic rats.
AU Gilbert J C; Takada T; Stein J E; Langer R; Vacanti J P
CS Department of Surgery, Children's Hospital, Boston, Massachusetts 02115.
SO TRANSPLANTATION, (1993 Aug) 56 (2) 423-7.
Journal code: 0132144. ISSN: 0041-1337.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199309
ED Entered STN: 19931008
Last Updated on STN: 19931008
Entered Medline: 19930917
AB Many severe metabolic deficiencies in children are caused by a single gene defect with a resultant single gene product deficiency. These diseases may be amenable to permanent cure using new techniques of gene transfer and cell transplantation. In many in vivo models of retroviral mediated **gene therapy**, a significant limiting factor is the ability to transplant a sufficient number of modified cells. To potentially circumvent this problem, we have developed a biodegradable polymer implant system capable of supporting large numbers of **genetically modified** cells. In this study, we inserted a reporter gene into syngeneic cultured normal fibroblasts and then transplanted these **genetically modified** cells into animals using synthetic biodegradable polymer fibers as temporary cell delivery scaffolds. To begin to develop a system capable of delivering desirable proteins secreted by **genetically modified** cells, Fischer 344 adult rat fibroblasts were transduced in tissue culture with a retrovirus containing the reporter gene Lac Z. These **genetically modified** cells (1.1 x 10(7) cells/graft) were then attached to the biodegradable polymer fibers and the polymer-cell graft was transplanted subdermally into syngeneic recipients (n = 9). There was persistence of the modified cells with expression of the reporter gene for at least 30 days. The estimated number of **genetically modified** cells per implanted graft decreased from a pretransplant value of 1.1 +/- 0.6 x 10(7) to 3.2 +/- 0.7 x 10(6) by 15 days after transplantation ($P < 0.01$). Thereafter, the cell number did not vary significantly to the conclusion of the study at day 30 (3.6 +/- 1.0 x 10(6) cells/graft). Evidence of ingrowth and incorporation of other **stromal** elements was present in the graft by 1 week post-transplantation, as judged by counterstained hematoxylin and eosin micrograph sections. Migration of modified cells to areas outside of the polymer-cell graft was not detected. Over the course of the study, there was little degradation of the polymer implant, although by day 30, evidence of early dissolution was evident. The number of polymer fibers per high power field increased slightly from 62.5 +/- 5.8 on day 1 to 77.3 +/- 26.6 on day 30 ($P > 0.2$). These data suggest that the use of biodegradable polymer fibers may permit the transplantation of **genetically modified** cells in sufficient numbers to deliver a therapeutically useful product. Polymer matrices allow for the attachment and site-specific transplantation of **genetically modified** cells.

L27 ANSWER 9 OF 20 MEDLINE DUPLICATE 4
AN 1999422063 MEDLINE
DN 99422063 PubMed ID: 10490771
TI Bone marrow **stromal** cells as a vehicle for gene transfer.
AU Ding L; Lu S; Batchu R; III R S; Munshi N
CS Central Arkansas Veterans Healthcare System, Myeloma and Transplantation Research Center, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA.
NC CA71092 (NCI)
HL55695 (NHLBI)
SO GENE THERAPY, (1999 Sep) 6 (9) 1611-6.
Journal code: 9421525. ISSN: 0969-7128.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200004
ED Entered STN: 20000413
Last Updated on STN: 20000413
Entered Medline: 20000403
AB Adoptive transfer of **genetically modified** somatic cells is playing an increasingly important role in the management of a wide spectrum of human diseases. Hematopoietic stem cells and lymphocytes have been used to transfer a variety of genes, however, they have limitations. In this study, the feasibility of retroviral gene transduction of bone marrow **stromal** cells, and the engraftment characteristics of these cells following infusion, was investigated in a murine transplantation model. **Stromal** cells derived from Balb/c mouse bone marrow were transduced with a replication-defective retrovirus containing the LacZ gene. Following three rounds of transduction, between 5 and 40% of the cells were positive for the LacZ gene. A total of 2 x 106 cells were infused into the same mouse strain. After the infusion, the LacZ gene was detected by PCR in the bone marrow, spleen, liver, kidney and lung; however, only the spleen and bone marrow samples were strongly positive. Quantitative PCR demonstrated that between 3 and 5% of spleen and bone marrow cells, and 1% of liver cells contained the LacZ gene at 3 weeks after infusion; <0.2% transduced cells were found in other organs. No difference was noted in engraftment between mice with or without irradiation before transplantation, suggesting that engraftment occurred without myeloablation. The infused transduced cells persisted for up to 24 weeks. Self-renewal of transplanted **stromal** cells was demonstrated in secondary transplant studies. Ease of culture and gene transduction and tissue specificity to hematopoietic organs (bone marrow, spleen, liver) is demonstrated, indicating that **stromal** cells may be an ideal vehicle for gene transfer.

L27 ANSWER 8 OF 20 MEDLINE DUPLICATE 3
AN 2000008963 MEDLINE
DN 20008963 PubMed ID: 10543618
TI Multipotential marrow **stromal** cells transduced to produce L-DOPA: engraftment in a rat model of Parkinson disease.
AU Schwarz E J; Alexander G M; Prockop D J; Azizi S A
CS Center for Gene Therapy, MCP Hahnemann University, Philadelphia, PA 19102, USA.
SO HUMAN GENE THERAPY, (1999 Oct 10) 10 (15) 2539-49.
Journal code: 9008950. ISSN: 1043-0342.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199911
ED Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991116
AB Bone marrow **stromal** cells can be used as an alternative source of cells for neural transplantation and repair. Here, the efficacy of **genetically modified** marrow **stromal** cells was examined in a rat model of Parkinson disease. Rat marrow **stromal** cells (rMSCs) and human marrow **stromal** cells (hMSCs) were genetically engineered by transduction with retroviruses encoding tyrosine hydroxylase (TH) and GTP cyclohydrolase I, the enzyme necessary for production of the tetrahydrobiopterin cofactor for TH (BH4). Transduced cells synthesized 3,4-dihydroxyphenylalanine (L-DOPA) in vitro and maintained their multipotentiality after retroviral transduction. To examine the cells in vivo, transduced rMSCs were injected into the striatum of 6-hydroxydopamine-lesioned rats. L-DOPA and metabolites were detected by microdialysis in the denervated striatum of rats that received doubly transduced rMSCs. Also, there was a significant reduction in apomorphine-induced rotation when compared with controls. The cells engrafted and survived for at least 87 days. However, expression of the transgenes ceased at about 9 days, an observation consistent with reports from other laboratories in which similar retroviruses were used to express transgenes in the brain.

L27 ANSWER 7 OF 20 MEDLINE DUPLICATE 2
AN 2001064092 MEDLINE
DN 20426147 PubMed ID: 10972331
TI In vivo expression of human growth hormone by **genetically modified** murine bone marrow **stromal** cells and its effect on the cells in vitro.
AU Suzuki K; Oyama M; Faulcon L; Robbins P D; Niyibizi C
CS Musculoskeletal Research Center, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, PA 15261, USA.
NC R29A42720
SO CELL TRANSPLANTATION, (2000 May-Jun) 9 (3) 319-27.
Journal code: 9208854. ISSN: 0963-6897.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200012
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001222
AB Human growth hormone (hGH) is frequently used clinically for growth abnormalities in children and also in adults with growth hormone deficiency. The hormone is usually administered to the individuals by frequent injections. In the present study we investigated the potential of bone marrow **stromal** cells as vehicles to deliver the GH in vivo by infusion of cells transduced with hGH cDNA into mice femurs. The effect of the hormone on the transduced cells in vitro was also assessed. Bone marrow **stromal** cells established from a mouse model of human osteogenesis imperfecta mice (oim) were transduced with a retrovirus containing hGH and neomycin resistance genes. The hGH-expressing cells were selected in a medium containing G418 and were then assessed for the hGH expression in vitro. The selected cells synthesized 15 ng/10(6) cells of hGH per 24 h in vitro and exhibited alkaline phosphatase activity when they were treated with the human recombinant bone morphogenetic protein 2 (rhBMP-2). The transduced cells also proliferated faster than the LacZ transduced cells but they did not exhibit a higher rate of matrix synthesis. When 2 x 10(6) hGH+ cells were injected into the femurs of mice, hGH was detected in the serum of the recipient mice up to 10 days after injection. The highest level of growth hormone expression, 750 pg/ml, was detected in the serum of the recipient mice I day after injection of the transduced cells. hGH was also detected in the medium conditioned by cells that were flushed from the femurs of the recipient mice at 1, 3, and 6 days after cell injection. These data indicate that bone marrow **stromal** cells could potentially be used therapeutically for the delivery of GH or any other therapeutic proteins targeted for bone. The data also suggest that GH may exert its effects on bone marrow **stromal** cells by increasing their rate of proliferation.

L27 ANSWER 4 OF 20 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 2002:261593 BIOSIS
DN PREV200200261593
TI Prolonged engraftment and transgene expression of **genetically modified** autologous bone marrow **stromal** (mesenchymal) cells after infusion post autotransplant: A platform for cell and **gene therapy**.
AU Keating, Armand (1); Filshie, Robin (1); Mollee, Peter (1); Wang, Xing-Hua (1)
CS (1) Princess Margaret Hospital/Ontario Cancer Institute and Toronto General Research Institute, University of Toronto, Toronto, ON Canada
SO Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 832a. http://www.bloodjournal.org/. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001
ISSN: 0006-4971.
DT Conference
LA English
AB In previous studies, we and others have demonstrated the engraftment of murine marrow **stromal** or mesenchymal cells (MSC) after intravenous infusion into conditioned or unconditioned hosts. Moreover, since these cells are readily transfected, the transplantation of **genetically modified** MSC may provide a suitable means of gene delivery for **gene therapy** protocols. As a first step towards this clinical goal, we performed a study exploring the feasibility, safety and engraftment of **genetically modified** MSC infused into three patients with delayed hematopoietic recovery after intensive therapy and autotransplant for hematologic malignancies. CD45(-) **stromal** cells derived from passaged adherent layers of long-term marrow cultures were electrotransfected with the pcDNA3/hFIX plasmid carrying the human Factor IX cDNA and the neo gene transcribed from the CMV immediate early and SV40 promoter, respectively. Between Sept 1998 and June 1999, autologous transfected MSC (50-90X106 cells) were infused without pre-conditioning into three patients with NHL, AML and HD 125, 49 and 210 days, respectively, after autotransplant. No significant toxicity was observed and the three patients remain alive and disease-free up to 33 months after MSC infusion. There was no immediate improvement in hematopoietic engraftment after the infusion. Gene marked cells were detected in all patients after MSC infusion by PCR for FIX cDNA using primers that span CMV promoter and hFIX sequences, and for neo, in marrow or cultured MSC. Furthermore, transcription of the hFIX transgene was detected in all patients using RT-PCR for the hFIX transgene. For example, in patient 2 (AML), PCR signals for both transgenes were detected in the nucleated marrow cells up to 6 months, and in MSC derived from a marrow aspirate at 8 months after **stromal** cell infusion, but not at 11 months. Also, the FIX transgene was transcriptionally active by RT-PCR in the nucleated marrow cells at 4 months, and in the MSC up to 6 months after infusion of the marked MSC. Pre-infusion non-transfected patient MSC were PCR and RT-PCR negative in all cases. Our data demonstrate durable engraftment of autologous marrow **stromal** cells in persons who did not receive conditioning immediately prior to MSC infusion. We have also shown that these **genetically modified** cells can be safely infused and express the transgene in vivo for up to 6 months. The results suggest that a phase II exploration of this approach in **gene therapy** protocols is warranted.

L27 ANSWER 3 OF 20 MEDLINE DUPLICATE 1
AN 2001462335 MEDLINE
DN 21398218 PubMed ID: 11506695
TI Baboon mesenchymal stem cells can be **genetically modified** to secrete human erythropoietin in vivo.
AU Bartholomew A; Patil S; Mackay A; Nelson M; Buyaner D; Hardy W; Mosca J; Sturgeon C; Siatskas M; Mahmud N; Ferrer K; Deans R; Moseley A; Hoffman R; Devine S M
CS Department of Surgery, University of Illinois at Chicago, Chicago, IL 60612, USA.
SO HUMAN GENE THERAPY, (2001 Aug 10) 12 (12) 1527-41.
Journal code: 9008950. ISSN: 1043-0342.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200111
ED Entered STN: 20010820
Last Updated on STN: 20011105
Entered Medline: 20011101
AB Human mesenchymal stem cells (MSCs) are capable of differentiating into multiple mesenchymal lineages including chondrocytes, osteocytes, adipocytes, and marrow **stromal** cells. Using a nonhuman primate model, we evaluated nonhuman primate MSCs as targets for **gene therapy**. Baboon MSCs (bMSCs) cultured from bone marrow aspirates appeared as a homogeneous population of spindle-shaped cells. bMSCs were capable of differentiating into adipocytes and osteocytes in vitro and chondrocytes in vivo. bMSCs were **genetically modified** with a bicistronic vector encoding the human erythropoietin (hEPO) gene and the green fluorescent protein (GFP) gene. Transduction efficiencies ranged from 72 to 99% after incubation of MSCs with retroviral supernatant. Transduced cells produced from 1.83 x 10(5) to 7.12 x 10(5) mIU of hEPO per 10(6) cells per 24 hr in vitro before implantation. To determine the capacity of bMSCs to express hEPO in vivo, transduced bMSCs were injected intramuscularly in NOD/SCID mice. In a separate experiment, transduced bMSCs were loaded into immunoisolatory devices (IIDs) and surgically implanted into either autologous or allogeneic baboon recipients. Human EPO was detected in the serum of NOD/SCID mice for up to 28 days and in the serum of five baboons for between 9 and 137 days. NOD/SCID mice experienced sharp rises in hematocrit after intramuscular injection of hEPO-transduced bMSCs. The baboon that expressed hEPO for 137 days experienced a statistically significant ($p < 0.04$) rise in its hematocrit. These data demonstrate that nonhuman primate MSCs can be engineered to deliver a secreted and biologically active gene product. Therefore, human MSCs may be an effective target for future human **gene therapy** trials.

L27 ANSWER 1 OF 20 MEDLINE
AN 2003009686 IN-PROCESS
DN 22403995 PubMed ID: 12516047
TI Bone marrow **stromal** cells as a genetic platformfor systemic delivery of therapeutic proteins in vivo: human factor IX model.
AU Krebsbach Paul H; Zhang Kezhong; Malik Ajay K; Kurachi Kotoku
CS University of Michigan School of Dentistry, Department of Oral Medicine, Pathology, and Oncology, Ann Arbor, Michigan, USA.
SO JOURNAL OF GENE MEDICINE, (2003 Jan-Feb) 5 (1) 11-7.
Journal code: 9815764. ISSN: 1099-498X.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20030108
Last Updated on STN: 20030108
AB BACKGROUND: Hemophilia B is an X-linked bleeding disorder that results from a deficiency in functional coagulation factor IX (hFIX). In patients lacking FIX, the intrinsic coagulation pathway is disrupted leading to a lifelong, debilitating and sometimes fatal disease. METHODS: We have developed an ex vivo **gene therapy** system using **genetically modified** bone marrow **stromal** cells (BMSCs) as a platform for sustained delivery of therapeutic proteins into the general circulation. This model exploits the ability of BMSCs to form localized ectopic ossicles when transplanted in vivo. BMSCs were transduced with MFG-hFIX, a retroviral construct directing the expression of hFIX. The biological activity of hFIX expressed by these cells was assessed in vitro and in vivo. RESULTS: Transduced cells produced biologically active hFIX in vitro with a specific activity of 90% and expressed hFIX at levels of approximately 497 ng/10(6) cells/24 h and 322 ng/10(6) cells/24 h for human and porcine cells, respectively. The secretion of hFIX was confirmed by Western blot analysis of the conditioned medium using a hFIX-specific antibody. Transduced BMSCs (8 x 10(6) cells per animal) were transplanted within scaffolds into subcutaneous sites in immunocompromised mice. At 1 week post-implantation, serum samples contained hFIX at levels greater than 25 ng/ml. Circulating levels of hFIX gradually decreased to 11.5 ng/ml at 1 month post-implantation and declined to a stable level at 6.1 ng/ml at 4 months. CONCLUSIONS: These findings demonstrate that **genetically modified** BMSCs can continuously secrete biologically active hFIX from self-contained ectopic ossicles in vivo, and thus represent a novel delivery system for releasing therapeutic proteins into the circulation.

L33 ANSWER 13 OF 13 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AN 1997-10813 BIOTECHDS
TI Using new obesity protein receptor(s) to treat weight disorders; human recombinant protein expression for use as an anorectic, antidiabetic or anticholesterolemic, or in obesity, diabetes, high blood lipid levels or high cholesterol level **gene therapy**
AU Chang M S; Welcher A A; Fletcher F A
PA Amgen
LO Thousand Oaks, CA, USA.
PI WO 9725424 17 Jul 1997
AI WO 1997-US128 2 Jan 1997
PRAI US 1996-774414 31 Dec 1996; US 1996-582825 4 Jan 1996
DT Patent
LA English
OS WPI: 1997-384981 [35]
AB A new human obesity protein (**OB**) receptor has a specified protein sequence, or a specified fragment, optionally with an N-terminal methionine residue. The protein may have a specified deletion, insertion or substitution mutation. DNA encoding the **OB** receptor, or a hybridizing or degenerate sequence, is also new. The DNA may be inserted in a virus or plasmid vector for expression in a prokaryote or eukaryote (especially human) host cell, optionally with modifications for enhanced expression. A new method of therapy of obesity, diabetes, high blood lipid levels or high cholesterol levels, or for cosmetic weight loss or weight maintenance, involves administration of the **OB** receptor protein, or an **OB** protein/**OB** receptor complex, or cells (e.g. recombinant cells) expressing the complex in vivo (optionally as separate populations expressing **OB** protein and **OB** receptor). (151pp)

L33 ANSWER 12 OF 13 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1998:121120 BIOSIS
DN PREV199800121120
TI Leptin **gene therapy** and daily protein administration;
A comparative study in the **ob/ob** mouse.
AU Morsy, M. A. (1); Gu, M. C.; Zhao, J. Z.; Holder, D. J.; Rogers, I. T.; Pouch, W. J.; Motzel, S. L.; Klein, H. J.; Gupta, S. K.; Liang, X.; Tota, M. R.; Rosenblum, C. I.; Caskey, C. T.
CS (1) Dep. Human Genetics, Merck and Co. Inc., WP26A-3000, Sumneytown Pike, West Point, PA 19486 USA
SO Gene Therapy, (Jan., 1998) Vol. 5, No. 1, pp. 8-18.
ISSN: 0969-7128.
DT Article
LA English
AB We have compared the efficacy of daily injection of recombinant leptin protein (rh-leptin) with adenovirus-mediated delivery of the murine or human leptin gene (Ad-leptin) for treatment of obesity in the **obese** (**ob/ob**) mouse model. We demonstrate an improved correction profile for obesity and associated surrogate markers using the adenovirus delivery method. Rate of weight loss and percentage satiety were significantly greater in the mice treated with Ad-leptin. These findings were associated with lower peak serum leptin levels with Ad-leptin (22.9 +- 2.6 ng/ml for the human gene, and 48.9 +- 11.5 ng/ml for the murine gene) compared to rh-leptin (385.2 +- 36.0 ng/ml). (Values are given as mean +- standard error of the mean.) Importantly, rh-leptin and **ex vivo**-expressed Ad-leptin were equivalently active in a functional cell-based assay. The primary difference in the two therapeutic approaches is the continuous chronic secretion of leptin mediated by gene delivery, versus the intermittent bolus delivery and rapid clearance of the daily injection of rh-leptin protein. Thus, in vivo findings suggest that leptin effects are better achieved at lower steady-state levels, a pharmacological feature attained here by **gene therapy**. These findings may have implications for the potential use of leptin in the treatment of obesity.

33 ANSWER 2 OF 13 MEDLINE
AN 1998197323 MEDLINE
DN 98197323 PubMed ID: 9536260
TI Leptin **gene therapy** and daily protein administration: a comparative study in the **ob/ob** mouse.
AU Morsy M A; Gu M C; Zhao J Z; Holder D J; Rogers I T; Pouch W J; Motzel S L; Klein H J; Gupta S K; Liang X; Tota M R; Rosenblum C I; Caskey C T
CS Department of Human Genetics, Merck and Co, Inc, West Point, PA 19486, USA.
SO GENE THERAPY, (1998 Jan) 5 (1) 8-18.
Journal code: 9421525. ISSN: 0969-7128.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199804
ED Entered STN: 19980422
Last Updated on STN: 20000303
Entered Medline: 19980415
AB We have compared the efficacy of daily injection of recombinant leptin protein (rh-leptin) with adenovirus-mediated delivery of the murine or human leptin gene (Ad-leptin) for treatment of obesity in the **obese** (**ob/ob**) mouse model. We demonstrate an improved correction profile for obesity and associated surrogate markers using the adenovirus delivery method. Rate of weight loss and percentage satiety were significantly greater in the mice treated with Adleptin. These findings were associated with lower peak serum leptin levels with Ad-leptin (22.9 +/- 2.6 ng/ml for the human gene, and 48.9 +/- 11.5 ng/ml for the murine gene) compared to rh-leptin (385.2 +/- 36.0 ng/ml). (Values are given as mean +/- standard error of the mean.) Importantly rh-leptin and **ex vivo**-expressed Ad-leptin were equivalently active in a functional cell-based assay. The primary difference in the two therapeutic approaches is the continuous chronic secretion of leptin mediated by gene delivery, versus the intermittent bolus delivery and rapid clearance of the daily injection of rh-leptin protein. Thus, in vivo findings suggest that leptin effects are better achieved at lower steady-state levels, a pharmacological feature attained here by **gene therapy**. These findings may have implications for the potential use of leptin in the treatment of obesity.

L39 ANSWER 96 OF 97 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AN 1993-04675 BIOTECHDS
TI Long-term expression of a retrovirally introduced beta-galactosidase gene in rodent cells **implanted** in vivo using biodegradable polymer meshes;
recombinant gene expression in mouse, rat with polyglycolic acid, polyglactin mesh **implant** carrying transformed mouse embryo fibroblast, rat femoral bone marrow **stromal** cell;
gene therapy
AU Naughton B A; Dai Y; Sibanda B; Scharfmann R; San Roman J; Zeigler F
CS Advan.Tissue-Sci.
LO Medical Laboratory Sciences Department, Hunter College School of Health Sciences, New York, New York, USA.
SO Somatic Cell Mol.Genet.; (1992) 18, 5, 451-62
CODEN: SCMGDN
DT Journal
LA English
AB The retro virus vector LNL-SLX-beta-gal, containing a 3.1 kb beta-galactosidase (EC-3.2.1.23) gene driven by the mouse DHFR promoter, was used to transfect C57BL/6J mouse 17-day-old embryonic fibroblast (EMF) primary cultures and 6-8-wk-old Long-Evans rat femoral bone marrow **stromal** (BMS) cells. Cell cultures containing more than 80% X-gal-positive cells were treated with a collagenase (EC-3.4.24.3)-dispase suspension and 5 million-50 million cells in 250 ul were inoculated onto 18 mm x 40 mm pieces of Dexon No.4 polyglycolic acid (PGA) mesh or Vicryl polyglactin (PGL) mesh. Mesh cultures were grown to confluence (about 2 wk) and 18 mm x 18 mm pieces of the mech were surgically **implanted** into adult male C57BL/J6 and Nu/Nu athymic mice (EMF) and 6-8-wk-old male Long-Evans rats (BMS). Beta-galactosidase activity was detected for up to 125, 123 and 90 days for EMF in nude mice, EMF in C57BL/J6 mice and BMS in Long-Evans rats, respectively. Non-infected cells grafted using the same methods did not stain with X-gal. The PGA and PGL meshes allowed **implantation** of cells at high density and induced rapid wound healing at the graft sites. (39 ref)

L39 ANSWER 87 OF 97 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 45
AN 95330899 EMBASE
DN 1995330899
TI **Ex vivo** expansion and subsequent infusion of human bone marrow-derived **stromal** progenitor cells (mesenchymal progenitor cells): Implications for therapeutic use.
AU Lazarus H.M.; Haynesworth S.E.; Gerson S.L.; Rosenthal N.S.; Caplan A.I.
CS Ireland Cancer Center, University Hospitals of Cleveland, Department of Medicine, 11100 Euclid Avenue,Cleveland, OH 44106, United States
SO Bone Marrow Transplantation, (1995) 16/4 (557-564).
ISSN: 0268-3369 CODEN: BMTRE
CY United Kingdom
DT Journal; Article
FS 016 Cancer
025 Hematology
LA English
SL English
AB We report a phase I trial to determine the feasibility of collection, **ex vivo** culture-expansion and intravenous infusion of human bone marrow-derived progenitor **stromal** cells (mesenchymal progenitor cells (MPCs)). Ten milliliter bone marrow samples were obtained from 23 patients with hematologic malignancies in complete remission. Bone marrow mononuclear cells were separated and adherent cells were culture-expanded in vitro for 4-7 weeks. Autologous MPCs were reinfused intravenously and a bone marrow examination repeated 2 weeks later for histologic assessment and in vitro hematopoietic cultures. Patient age ranged from 18 to 68 years and 12 subjects previously had undergone an autologous or syngeneic bone marrow transplant 4-52 months prior to collection of MPCs. A median of 364 x 106 nucleated bone marrow cells (range: 103 to 1004 x 106) were used for **ex vivo** expansion. Median number of MPCs which were obtained after **ex vivo** culture expansion was 59.0 (range: 1.1 to 347 x 106) representing a median cell doubling of 16000-fold (13 doublings). Fifteen of 23 patients completed the **ex vivo** expansion and underwent MPC infusion. Time to infusion of MPCs after collection ranged from 28 to 49 days. Five patients in each of three groups were given 1, 10 and 50 x 106 MPCs. No adverse reactions were observed with the infusion of the MPCs. MPCs obtained from cancer patients can be collected, expanded in vitro and infused intravenously without toxicity. Future trials will address the potential of these cells to regenerate the bone marrow microenvironment, enhance recovery of blood counts when given in conjunction with autologous peripheral blood progenitor cell transplantation and examine their utility as targets for **gene therapy**.

L39 ANSWER 77 OF 97 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AN 1996-08198 BIOTECHDS
TI Three dimensional culture of liver cells;
liver cell culture in culture vessel for biologically active molecule production, and transformation for **gene therapy**
AU Naughton B A; Naughton G K
PA Advan.Tissue-Sci.
LO La Jolla, CA, USA.
PI US 5510254 23 Apr 1996
AI US 1994-241259 11 May 1994
PRAI US 1994-241259 11 May 1994
DT Patent
LA English
OS WPI: 1996-221250 [22]
AB A method for culturing liver cells in vitro comprises (a) inoculating liver parenchymal cells onto a living **stromal** tissue prepared in vitro, comprising **stromal** cells and connective tissue proteins naturally secreted by the **stromal** cells attached to and enveloping a framework of non-living biocompatible material formed into a three-dimensional structure having interstitial spaces bridged by the **stromal** cells, and (b) incubating the inoculated tissue in a nutrient medium so that the liver cells proliferate. The **stromal** cells are fibroblasts or a combination of fibroblasts and endothelial cells, pericytes, macrophages, monocytes, leukocytes, plasma cells, mast cells or adipocytes. The framework is a mesh of (non-) biodegradable material, and may be precoated with collagen. The cultures can be used as **implants**, for screening of cytotoxic agents or drugs, for production of biologically active molecules in culture vessels, for production of extracorporeal liver-assisted devices, etc. The cells can also be genetically transformed and used for **gene therapy**. (24pp)

L39 ANSWER 94 OF 97 MEDLINE DUPLICATE 48
AN 93362170 MEDLINE
DN 93362170 PubMed ID: 8356600
TI Cell transplantation of genetically altered cells on biodegradable polymer scaffolds in syngeneic rats.
AU Gilbert J C; Takada T; Stein J E; Langer R; Vacanti J P
CS Department of Surgery, Children's Hospital, Boston, Massachusetts 02115.
SO TRANSPLANTATION, (1993 Aug) 56 (2) 423-7.
Journal code: 0132144. ISSN: 0041-1337.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199309
ED Entered STN: 19931008
Last Updated on STN: 19931008
Entered Medline: 19930917
AB Many severe metabolic deficiencies in children are caused by a single gene defect with a resultant single gene product deficiency. These diseases may be amenable to permanent cure using new techniques of gene transfer and cell transplantation. In many in vivo models of retroviral mediated **gene therapy**, a significant limiting factor is the ability to transplant a sufficient number of modified cells. To potentially circumvent this problem, we have developed a biodegradable polymer **implant** system capable of supporting large numbers of genetically modified cells. In this study, we inserted a reporter gene into syngeneic cultured normal fibroblasts and then transplanted these genetically modified cells into animals using synthetic biodegradable polymer fibers as temporary cell delivery scaffolds. To begin to develop a system capable of delivering desirable proteins secreted by genetically modified cells, Fischer 344 adult rat fibroblasts were transduced in tissue culture with a retrovirus containing the reporter gene Lac Z. These genetically modified cells (1.1 x 10(7) cells/graft) were then attached to the biodegradable polymer fibers and the polymer-cell graft was transplanted subdermally into syngeneic recipients (n = 9). There was persistence of the modified cells with expression of the reporter gene for at least 30 days. The estimated number of genetically modified cells per **implanted** graft decreased from a pretransplant value of 1.1 +/- 0.6 x 10(7) to 3.2 +/- 0.7 x 10(6) by 15 days after transplantation ($P < 0.01$). Thereafter, the cell number did not vary significantly to the conclusion of the study at day 30 (3.6 +/- 1.0 x 10(6) cells/graft). Evidence of ingrowth and incorporation of other **stromal** elements was present in the graft by 1 week post-transplantation, as judged by counterstained hematoxylin and eosin micrograph sections. Migration of modified cells to areas outside of the polymer-cell graft was not detected. Over the course of the study, there was little degradation of the polymer **implant**, although by day 30, evidence of early dissolution was evident. The number of polymer fibers per high power field increased slightly from 62.5 +/- 5.8 on day 1 to 77.3 +/- 26.6 on day 30 ($P > 0.2$). These data suggest that the use of biodegradable polymer fibers may permit the transplantation of genetically modified cells in sufficient numbers to deliver a therapeutically useful product. Polymer matrices allow for the attachment and site-specific transplantation of genetically modified cells.

L39 ANSWER 73 OF 97 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.
AN 1998:67361 BIOSIS
DN PREV199800067361
TI **Ex vivo gene therapy** of hemophilia A and B using bone marrow **stromal** cells in a canine model.
AU Hurwitz, D. R.; Chiang, G. G.; Cherington, V.; Rubin, H.; Wang, T.; Sobolewski, J.; Galanopoulos, T.; Natale, A.; McGrath, C. A.; Bizinkauskas, C. B.; Merrill, W.; Hansen, M.; Levine, P. H. (1); Greenberger, J. S.
CS (1) Meml. Health Care, Worcester, MA USA
SO Blood, (Nov. 15, 1997) Vol. 90, No. 10 SUPPL. 1 PART 1, pp. 239A. Meeting Info.: 39th Annual Meeting of the American Society of Hematology San Diego, California, USA December 5-9, 1997 The American Society of Hematology . ISSN: 0006-4971.
DT Conference
LA English

L39 ANSWER 74 OF 97 MEDLINE DUPLICATE 37
AN 97169875 MEDLINE
DN 97169875 PubMed ID: 9017418
TI Systemic delivery of human growth hormone or human factor IX in dogs by reintroduced genetically modified autologous bone marrow **stromal** cells.
AU Hurwitz D R; Kirchgesser M; Merrill W; Galanopoulos T; McGrath C A; Emami S; Hansen M; Cherington V; Appel J M; Bizinkauskas C B; Brackmann H H; Levine P H; Greenberger J S
CS ALG Company, Marlboro, MA 01752, USA.
SO HUMAN GENE THERAPY, (1997 Jan 20) 8 (2) 137-56. Journal code: 9008950. ISSN: 1043-0342.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199706
ED Entered STN: 19970612
Last Updated on STN: 19970612
Entered Medline: 19970603
AB Canine bone marrow **stromal** cells were expanded to numbers in excess of 10(9) cells from the initial 10-20 ml of marrow aspirates and transfected to express high levels of human growth hormone (hGH) in vitro. **Ex vivo**-modified marrow **stromal** cells were used in a **gene therapy** model system for the systemic delivery of transgene products in dogs. Adherent bone marrow **stromal** cell cultures, established and expanded from iliac crest marrow aspirates from each of 8 dogs, were transfected with a hGH gene plasmid expression vector and shown to express from 0.54-3.84 micrograms/10(6) cells per 24 hr hGH in vitro. The transfected plasmid vector does not possess a eukaryotic origin of replication nor does it possess sequences required for efficient integration into the host cell genome. As such, expression was expected to be transient. Transfected cells were autologously reintroduced into each dog by either infusion into a foreleg vein or directly into iliac crest marrow. In two cases, the **stromal** cells were cryopreserved following transfection, and subsequently thawed and infused. In one case, the expanded **stromal** cells were first cryopreserved, and then thawed, recultured, transfected, and infused. Reintroduced cell numbers ranged from 2.2 x 10(7) to 2.6 x 10(9), with total hGH expression capacities ranging from 62 to 1,400 micrograms/24 hr. Plasma of each of the dogs contained detectable hGH for a mean of 3.1 days (SD +/- 0.8 day) ranging from 2 to 5 days following reinfusion of cells. Peak plasma levels ranged from 0.10 to 1.76 ng/ml.

Similar hGH expression values, based upon total expression capacity of the cells infused and dog body weight, were obtained for all dogs. Vector-modified **stromal** cells were detectable, by polymerase chain reaction (PCR) analysis, in the peripheral circulation following reinfusion in all 4 dogs analyzed. In 3 of the dogs, modified **stromal** cells were detected for 8.5-15 weeks. In addition, modified **stromal** cells were detected in iliac crest marrow of 2 dogs for 9 and 13 weeks, respectively, following reinfusion. In another experiment, cultured bone marrow **stromal** cells were transfected with a human factor IX (hFIX) plasmid vector. Modified cells (5.57 x 10(8)), with a total hFIX expression capacity of 281 micrograms/24 hr, were reinfused, resulting in detectable hFIX in plasma continuously for 9 days with a peak level of 8 ng/ml on day 1. These results demonstrate that the **ex vivo** bone marrow **stromal** cell system is a potentially powerful method by which to deliver secreted transgene product to the systemic circulation of large animals.

L39 ANSWER 62 OF 97 MEDLINE DUPLICATE 28
AN 1998167472 MEDLINE
DN 98167472 PubMed ID: 9508053
TI Bone marrow **stromal** cells as targets for **gene therapy** of hemophilia A.
AU Chuah M K; Brems H; Vanslembrouck V; Collen D; Vandendriessche T
CS Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology-University of Leuven, Belgium.
SO HUMAN GENE THERAPY, (1998 Feb 10) 9 (3) 353-65.
Journal code: 9008950. ISSN: 1043-0342.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; AIDS
EM 199804
ED Entered STN: 19980430
Last Updated on STN: 19990129
Entered Medline: 19980421
AB Attempts to develop an **ex vivo gene therapy** strategy for hemophilia A, using either primary T cells or bone marrow (BM) stem/progenitor cells have been unsuccessful, due to the inability of these cell types to express coagulation factor VIII (FVIII). As an alternative, we evaluated the potential of BM-derived **stromal** cells which can be readily obtained and expanded in vitro. Human and murine BM **stromal** cells were transduced with an intron-based Moloney murine leukemia virus (MoMLV) retroviral vector expressing a B-domain-deleted human factor VIII cDNA (designated as MFG-FVIIIdeltaB). Transduction efficiencies were increased 10- to 15-fold by phosphate depletion and centrifugation, which obviated the need for selective enrichment of the transduced BM **stromal** cells. This resulted in high FVIII expression levels in transduced human (180 +/- 4 ng FVIII/10[6] cells per 24 hr) and mouse (900 +/- 130 ng FVIII/10[6] cells per 24 hr) BM **stromal** cells. Pseudotyping of the MFG-FVIIIdeltaB retroviral vectors with the gibbon ape leukemia virus envelope (GALV-env) resulted in significantly higher transduction efficiencies (100 +/- 20%) and FVIII expression levels (390 +/- 10 ng FVIII/10[6] cells per 24 hr) in transduced human BM **stromal** cells than with standard amphotropic vectors. This difference in transduction efficiency correlated with the higher titer of the GALV-env pseudotyped viral vectors and with the higher GALV receptor (GLVR-1) versus amphotropic receptor (GLVR-2) mRNA expression levels in human BM **stromal** cells. These findings demonstrate the potential of BM **stromal** cells for **gene therapy** in general and hemophilia A in particular.

L39 ANSWER 37 OF 97 MEDLINE DUPLICATE 16
AN 2000456805 MEDLINE
DN 20443074 PubMed ID: 10987005
TI **Ex vivo gene therapy** to produce bone using different cell types.
AU Musgrave D S; Bosch P; Lee J Y; Pelinkovic D; Ghivizzani S C; Whalen J; Niyibizi C; Huard J
CS Department of Orthopaedic Surgery, University of Pittsburgh, PA, USA.
NC 1P60 AR44811-01 (NIAMS)
SO CLINICAL ORTHOPAEDICS AND RELATED RESEARCH, (2000 Sep) (378) 290-305. Journal code: 0075674. ISSN: 0009-921X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 200009
ED Entered STN: 20001005
Last Updated on STN: 20001005
Entered Medline: 20000928
AB **Gene therapy** and tissue engineering promise to revolutionize orthopaedic surgery. This study comprehensively compares five different cell types in **ex vivo gene therapy** to produce bone. The cell types include a bone marrow **stromal** cell line, primary muscle derived cells, primary bone marrow **stromal** cells, primary articular chondrocytes, and primary fibroblasts. After transduction by an adenovirus encoding for bone morphogenetic protein-2, all of the cell types were capable of secreting bone morphogenetic protein-2. However, the bone marrow **stromal** cell line and muscle derived cells showed more responsiveness to recombinant human bone morphogenetic protein-2 than did the other cell types. In vivo injection of each of the cell populations transduced to secrete bone morphogenetic protein-2 resulted in bone formation. Radiographic and histologic analyses corroborated the in vitro data regarding bone morphogenetic protein-2 secretion and cellular osteocompetence. This study showed the feasibility of using primary bone marrow **stromal** cells, primary muscle derived cells, primary articular chondrocytes, primary fibroblasts, and an osteogenesis imperfecta **stromal** cell line in **ex vivo gene therapy** to produce bone. The study also showed the advantages and disadvantages inherent in using each cell type.

L39 ANSWER 25 OF 97 EMBASE COPYRIGHT 2003 ELSEVIER SCI. B.V.DUPLICATE 12
AN 2001229300 EMBASE
TI Stem cell transplantation and **gene therapy** in utero.
AU Surbek D.V.; Schatt S.; Holzgreve W.
CS Dr. W. Holzgreve, Universitats-Frauenklinik, Kantonsspital Basel, Schanzenstrasse 46, CH-4031 Basel, Switzerland
SO Infusionstherapie und Transfusionsmedizin, (2001) 28/3 (150-158).
Refs: 110
ISSN: 1424-5485 CODEN: IRANEE
CY Switzerland
DT Journal; General Review
FS 007 Pediatrics and Pediatric Surgery
022 Human Genetics
025 Hematology
LA English
SL English; German
AB Background: Allogeneic hematopoietic stem cell transplantation in utero has been successfully used for the prenatal treatment of severe combined immunodeficiency syndrome. However, this therapy has not been successful in the treatment of other conditions in which the fetus is immunologically competent. Material and Methods: We reviewed the currently explored strategies to overcome these problems, including prenatal **gene therapy** using **ex vivo** transduced autologous hematopoietic cells or direct gene targeting in utero. Results: Some of the strategies such as **stromal** cell co-transplantation have been shown to be successful in preclinical studies. Similarly, prenatal gene transfer has been shown to be feasible in the fetal sheep model; however, safety concerns regarding transduction of fetal germ cells or maternal cells remain. Conclusion: Progress is being made in the exploration of new modalities of in utero transplantation although the procedure remains experimental and long-term clinical efficacy needs to be proven. In utero **gene therapy** seems feasible, but more animal studies are needed in order to assess its safety.

L39 ANSWER 16 OF 97 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT AND ISI
AN 2002-03517 BIOTECHDS
TI Treating demyelinizing and neurodegenerative diseases, e.g. Alzheimer's disease, comprises **implanting** cells that differentiated from mesenchymal **stromal** cells;
the use of human differentiated cell in **gene therapy**
AU Tennekoon G; Cole A J; Grinspan J; Beesley J S
PA Child.Hosp.Philadelphia
LO Philadelphia, PA, USA.
PI WO 2001078753 25 Oct 2001
AI WO 2001-US12002 12 Apr 2001
PRAI US 2000-196473 12 Apr 2000
DT Patent
LA English
OS WPI: 2002-017559 [01]
AB A method for treating diseases characterized by damaged myelin or neurological deterioration is claimed. It involves compensating for these conditions by using cells (A) that have differentiated from mesenchymal **stromal** cells (MSC), where a composition containing MSC and carrier is prepared in vitro, then exposed to conditions that cause differentiation to neurons or oligodendrocytes. Also claimed are: preparing differentiated cells (neurons or oligodendrocytes) by exposing an MSC or carrier composition to differentiation-inducing condition in vitro; composition (B) consisting essentially of immortalized MSC and carrier, optionally also one or more exogenous genes; and differentiating MSC into an oligodendrocyte precursor and mature oligodendrocytes. The method is used to treat disease characterized by loss of neurons, e.g. Parkinson disease, Alzheimer disease, Huntington disease, stroke or head trauma, or dysfunction in ganglioside storage or demyelinization, e.g. Tay-Sachs syndrome, multiple sclerosis etc. Where MSC are transfected with exogenous genes, they can be used as vectors for **gene therapy**, for treating brain cancer. (25pp)